Biochemical and Biophysical Research Communications 435 (2013) 597–602

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Resveratrol improves learning and memory in normally aged mice through microRNA-CREB pathway

Yong-Na Zhao [a,b], Wei-Feng Li [a,b], Fei Li [a,b], Zhen Zhang [a,b], Yun-Dong Dai [a,b], Ai-Li Xu [c], Cui Qi [a], Ji-Min Gao [b,*], Jun Gao [a,*]

[a] Model Animal Research Center of Nanjing University and MOE Key Laboratory of Model Animals for Disease Study, 12 Xuefu Road, Pukou High-Tech Zone, Nanjing 210061, China
[b] Zhejiang Provincial Key Laboratory for Technology and Application of Model Organisms, School of Life Sciences, Wenzhou Medical University, Zhejiang 325035, China
[c] Department of Physiology, Binzhou Medical University, Shandong 264003, China

ARTICLE INFO

Article history:
Received 3 May 2013
Available online 16 May 2013

Keywords:
Resveratrol
Learning and memory
Aging
Sirtuin
Micro-RNA
CREB
BDNF

ABSTRACT

Resveratrol (RSV) is a natural compound found in grapes and red wine. It has been well known for its beneficial effects as a dietary supplement in prevention of cardiovascular diseases and cancer. Recently, *in vitro* studies have reported the neuroprotective role of RSV in neurodegenerative process in Alzheimer's disease (AD). However, *in vivo* effects of RSV on the decline of brain function accompanying the aging process, especially those on cognitive loss, have not been not investigated. Here we report that, after intraventricular injection of RSV for one week in 8–9 month-old mice, the long-term memory formation and the LTP induction from hippocampus CA1 were improved. The RSV enhancement effects were blocked in *SIRT1* mutant mice. Additional experiments suggest that RSV effects are likely to be mediated through reduced expressions of miR-134 and miR-124, which may in turn up-regulate CREB levels to subsequently promote BDNF synthesis. These findings demonstrate a role for RSV in cognition and a microRNA-CREB-BDNF mechanism by which RSV regulates these processes, demonstrating its value as a potential therapeutic target against CNS disorders in aging.

1. Introduction

The industrialized world's population is growing increasingly older. The physiological process of aging involves a progressive cognitive loss caused by deterioration of brain function, which includes a decrease in learning and memory skills and slower responses to intellectual stimuli. In age-related neuronal disorders, the free radicals and the oxidative stress they generate are known as the prime candidates responsible for producing the cell changes in such diseases [1,2]. The free radical hypothesis of aging has been utilized to explain the increased incidence of cancer and heart disease. The brain may be even more vulnerable to oxidative stress, as it exhibits reduced free radical scavenging ability and utilizes high amounts of oxygen [3,4]. Recently, a number of studies from the diets rich in fruit and vegetables or herbal extracts that exhibit anti-oxidant activities have shown some positive effects on reducing certain types of cancer and cardiovascular diseases [5–7], suggesting that these kinds of dietary modifications may also be beneficial in slowing the neuronal/behavioral deficits associated with aging.

Numerous contents within dietary fruits and vegetables exhibit anti-aging activities in various systems, among which one component resveratrol (RSV) (3,4′,5-trihydroxystilbene) is such a promising candidate [7]. RSV is a dietary polyphenol present in more than 70 different plants, especially in skin of grapes, and is speculated to be responsible for the "French Paradox" [8,9]. RSV exists in two isomers: *trans*- and *cis*-resveratrol, which may have different biological effects. It has been known that *trans*-resveratrol is nontoxic and is most widely studied. The number of studies is growing to show RSV's various beneficial biological effects, including its antioxidant, anti-inflammatory activities, release of neurotransmitters and neuromodulators [10–14]. Moreover, pre-clinical studies have demonstrated that RSV has the ability to limit the pathological states associated with cerebral ischemic injury, diabetes, arthritis, and a number of other aging-associated disorders [15–18]. Mechanistically, RSV has also been shown to mimic a calorie-restriction diet that extends lifespan and stress resistance by linking to the longevity gene Silent Information Regulator T1 (SIRT1) in yeast, worms, and flies [19,20]. Recent studies suggest functional relevance of SIRT1

* Corresponding authors at: Provincial Key Laboratory for Technology and Application of Model Organisms, School of Life Sciences, Wenzhou Medical University, Zhejiang 325035, China. Fax: 0577-86689748 (J.-M. Gao). Model Animal Research Center of Nanjing University and MOE Key Laboratory of Model Animals for Disease Study, 12 Xuefu Road, Pukou High-Tech Zone, Nanjing 210061, China. Fax: +86-025-58641500 (J. Gao).
E-mail addresses: jimingao@yahoo.com (J.-M. Gao), jungao@nju.edu.cn (J. Gao).

0006-291X
http://dx.doi.org/10.1016/j.bbrc.2013.05.025

activity in the brain [21,22]. Activation of SIRT1 is neuroprotective in a Cdk5 model of neurodegeneration [10]. Our previous data showed that SIRT1 dysfunction contributed to impaired learning and memory through microRNA-CREB-BDNF pathway [22].

Aging and age-associated neurodegenerative diseases are accompanied with various degrees of behavioral impairments including impaired learning and memory. And RSV ameliorated cognitive decline in Huntington's disease model mice or senescence-accelerated mice [23–25]. Whether RSV can improve normal behavior or brain function in a normal, healthy elderly is unknown. In the present study, we investigated the effect of RSV on learning and memory through intracerebroventricular injection of RSV into 8–9 month-old mice. And we observed a positive effect of RSV on memory formation and LTP induction in these aged mice. Furthermore, RSV treatment of cultured hippocampus slices causes reduction of several CREB-targeting miRNAs and a known CREB target, BDNF, which is consistent with an increase of CREB protein in hippocampus tissue from RSV-treated mice.

2. Materials and methods

2.1. Mice

Animals were derived from the C57BL/6J strain obtained from Model Animal Research Center. And the *SIRT1*$^{\Delta ex4}$/Nestin-Cre mice were kindly provided by Dr. Tsai LH (Massachusetts Institute of Technology, Cambridge, MA). All animal protocols are approved by the Animal Care and Use Committee of the Model Animal Research Center, the host for the National Resource Center for Mutant Mice in China, Nanjing University.

2.2. Fear conditioning tests

Training consists of a 3 min exposure of mice to the conditioning box (context) followed by a tone [30 s, 20 kHz, 75 dB sound pressure level (SPL)] and a foot shock (2 s, 0.8 mA, constant current). The memory test was performed 24-h later or 48-h by re-exposing the mice for 3 min into the conditioning context or to a tone (20 kHz, 75 dB SPL) in a novel context. Freezing, defined as a lack of movement except for breathing associated with a crouching posture, was recorded every 10 s by two trained observers (one was unaware of the experimental conditions) during 3 min (a total of 18 sampling intervals). The number of observations indicating freezing obtained as a mean from both observers was expressed as a percentage of the total number of observations.

2.3. Electrophysiological analysis

The field excitatory postsynaptic potentials (field EPSP [fEPSP]) from hippocampal CA1 region were recorded as described previously [22]. Briefly, transverse hippocampal slices (400 μm thick) were prepared from 8 to 9 month-old male mice. After decapitation, the brain was removed and placed in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) at 4 °C. Slices were cut with a Leica VT1000S vibratome (Leica instruments Ltd., Wetzlar, Germany) and maintained at 32 °C in a holding chamber filled with oxygenated ACSF for 1-h. After an equilibration period of at least 2 h at room temperature, a single slice was transferred to the recording chamber, where it was held between two nylon nets and continuously perfused with oxygenated ACSF (23–25 °C) at a flow rate of 2.5–3 ml/min.

2.4. Cannulation and injections

Double cannulae were implanted 7 days before the experiments, under anesthesia. For resveratrol injection, the cannulae were placed in lateral brain ventricles, AP-0.5 mm, lateral 1 mm, depth 2 mm. Resveratrol (5 μg/μl, Sigma) or vehicle was injected bilaterally for a week using a microinjector (CMA/microdialysis) over a 1-min period, so that a volume of 0.5 μl was injected into each side. Resveratrol (25% DMSO/artificial cerebrospinal fluid) was prepared fresh immediately before each injection.

2.5. Protein extraction and immunoblotting

The hippocampi were collected and lysesed in RIPA buffer. The lysates were incubated for 15 min on ice and centrifuged for 15 min at 4,000×*g* at 4 °C. The supernatant was collected. The lysates were subjected to 10% SDS–PAGE followed by immunoblotting.

2.6. Taqman® miRNA real time qPCR

Total small RNA was extracted using PureLinK miRNA Isolation Kit (Invitrogen) according to the manufacturer's instructions. RNA was reverse-transcribed using specific miRNA stem-loop primers and the Taqman® microRNA reverse transcription kit (Applied Biosystems). Mature miRNA expression was measured with Taqman® microRNA assays (Applied Biosystems) according to the manufacturer's instructions.

3. Results

3.1. RSV improves memory formation in mice

To investigate the effect of RSV on learning and memory, mice were treated by RSV through cerebroventricular injection for 1-week. Firstly, we examined whether the acetylation of PGC-1alpha, a known target for SIRT1, was decreased following RSV treatment. Indeed, we observed a decrease in acetylation of PGC-1alpha in hippocampus (Fig. 1A), suggesting that SIRT1 activity is increased following RSV treatment. Then, we investigated that the effect of RSV on memory formation. Eight to nine month-old mice were trained using Pavlovian fear conditioning paradigms prior to a memory test 24 h later. Mice treated with RSV for 1-week ($n = 10$) displayed an increased freezing level in both the context- and tone-dependent fear learning (Fig. 1B, left and right, respectively) compared with that of the control mice ($n = 10$). This was not due to changes in locomotors activity or exploratory behavior (Fig. 1D). Moreover, in a striking contrast to control mice, RSV did not enhance freezing behavior in neuron-specific SIRT1-deficient mice (*SIRT1*Δ) (Fig. 1C), which demonstrates a SIRT1-dependent mode of action for RSV. As a control, neither RSV nor the status of SIRT1 affected the shock sensitivity of the mice (Fig. 1D). These results indicate that RSV acts upon SIRT1 to play a critical regulatory role on memory formation in aged mice.

3.2. RSV facilitates LTP induction in hippocampus slices

Acutely dissociated hippocampus slices were treated with RSV for 1-h before and during electrophysiological recording. RSV treatment did not change the fEPSP baseline (Fig. 2A). Input–output coupling (Fig. 2B) was not significantly different between RSV and Veh mice. However, RSV treatment facilitated an LTP response from one train of θ burst stimulation (1 × TBS) in hippocampal slices prepared from control mice (Fig. 2C). Moreover, RSV did not facilitate LTP induction in *SIRT1*Δ mice (Fig. 2D). These results demonstrate a SIRT1-dependent mode of action for RSV on synaptic plasticity.

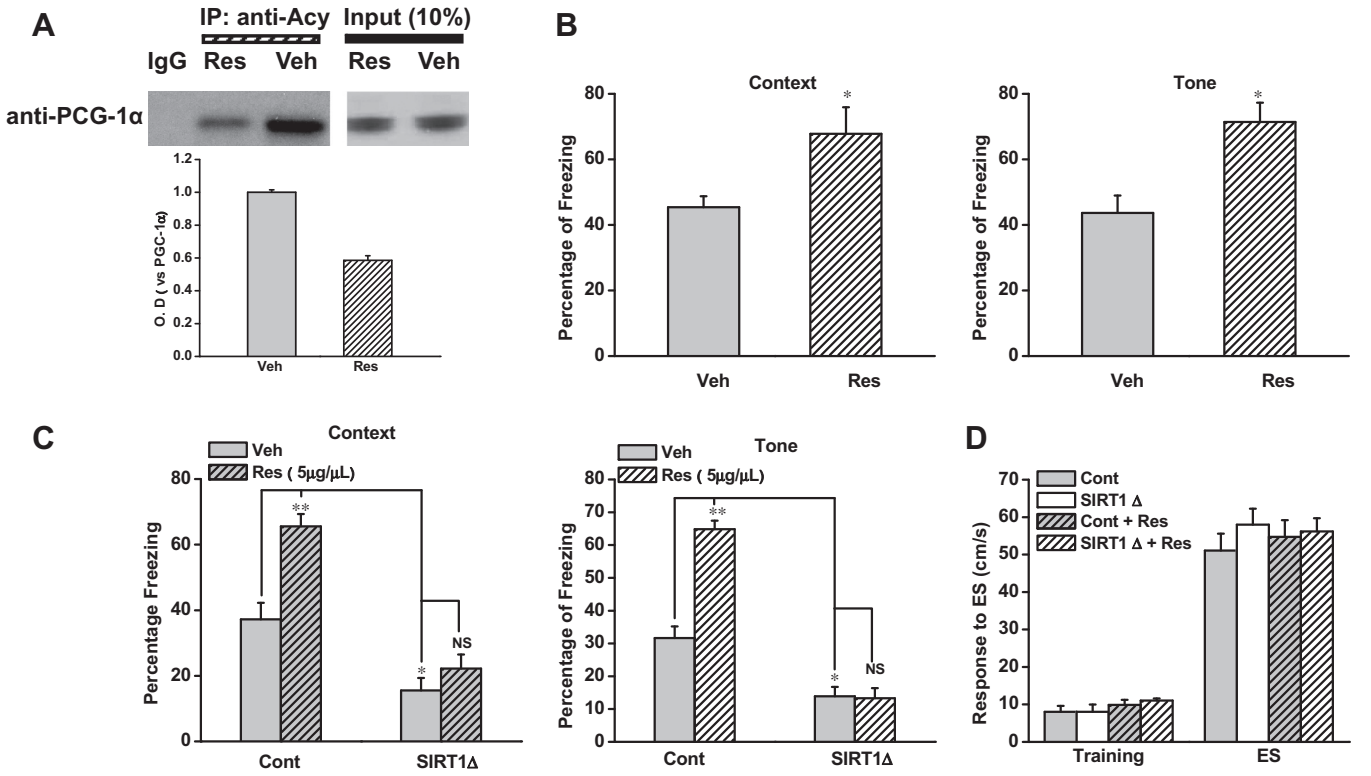

Fig. 1. Resveratrol, a SIRT1 agonist, improves associative learning by targeting SIRT1. (A) Acetylation levels of PGC-1alpha was decreased in animals treated with resveratrol (RSV, daily ICV injection, 5 μg/μL, 0.5 μL, for 1 week), compared to animals injected with vehicle (Veh). (B) Mice subjected to injection of RSV displayed significantly improved freezing behavior during the contextual and tone-dependent memory test compared to the vehicle group (RSV, $n = 10$; Veh, $n = 10$). (C) Freezing behavior was reduced in *SIRT1* mutant (*SIRT1*Δ) mice compared with floxed littermate control (Cont) mice (*SIRT1*Δ, $n = 10$; Cont, $n = 10$). Moreover, RSV ICV treatment for a week did not display enhanced freezing behavior in *SIRT1*Δ mice, in contrast to control mice (*SIRT1*Δ, $n = 10$; Cont, $n = 10$). (D) The response to ES at baseline, and after training, did not differ between control and *SIRT1*Δ mice, with and without RSV treatment.

3.3. RSV enhances the level of miR-124 and miR-134

It has been reported that SIRT1 regulates learning and memory through miR134 [22]. Thus, we evaluated the expression of microRNAs using mature miRNA-specific quantitative PCR (TaqMan MicroRNA assay). Consistent with the previor data, our qPCR analyses confirmed significant changes in expression of miR-134 after hippocampus slices was treated with 5 μM RSV for 1-h (Fig. 3A). Moreover, the level of miR-124 was also decreased after RSV treatment (Fig. 3A). Both of these two microRNAs were shown to regulate CREB expression and activity [26,27]. Thus, we further investigated the effect of RSV on CREB activity using a luciferase reporter containing CRE-binding elements in cultured CAD cells. As shown in Fig. 3B, CREB activity was dose-dependently enhanced by RSV treatment in CAD cells. Moreover, we observed the toxic effect of RSV on cultured CAD cells in 50 μM.

3.4. RSV treatment increases the expression of CREB and BDNF in acutely dissociated hippocampus slices

Next, we investigated if RSV regulates the expression of CREB. As shown in Fig. 4A, the expression level of CREB was significantly increased in hippocampi of mice treated with RSV for 1-week. Moreover, the level of BDNF, a CREB downstream target, was also enhanced in RSV treated slices (Fig. 4B). Owing to the importance of CREB-BDNF axis in cognitive function, our data collectively suggest the role of this functional axis downstream of a previously identified SIRT1-miRNA pathway in mediating RSV's positive effect on learning and memory in aged mice.

4. Discussion

In our study, resveratrol (RSV) significantly improved memory formation and synaptic plasticity compared with the control 8–9 month-old mice treated with Veh. And its effect is SIRT1-dependant since the latter positive effects of RSV is blocked in *SIRT1* mutant mice. Moreover, after RSV treatment in cultured hippocampi slices, the levels of miR-134 and 124 were downregulated, which accompanied an increase of BDNF. Together with the data that CREB protein is increased in the hippocampus of RSV-treated mice, our results collectively suggested that RSV acts via an age-independent miR-CREB-BDNF pathway to improve learning and memory and that a RSV-rich diet may be beneficial for preserving cognitive function by in aged individuals.

Resveratrol is a polyphenol phytoalexin which is synthesized by various edible plants in response to environmental stress (such as heavy metal ions) or fungal infection [6]. It has been well known that RSV has antioxidant, anti-flammatory, cardioprotective and anti-carcinogenic effects [5,7,11]. Moreover, RSV has been recently shown neuroprotective effects. For example, RSV administration has been effective in reducing damage in a number of cell cultures and animal models of central nervous system ischemia, diminishing the toxicity induced by amyloid β peptide [28–30], and preventing kainic acid induced excitotoxicity [31]. Furthermore, more data showed that RSV improved the impaired learning and memory in neurodegenerative conditions such as that in AD or HD models, or cognitive impairment induced by scopolamine or prenatal stress [32,33]. In this study, we investigated the role of RSV on learning and memory in normally aged mice. After one-week treatment of RSV, the locomotor and exploratory activities of mice were not affected as no any significant differences were

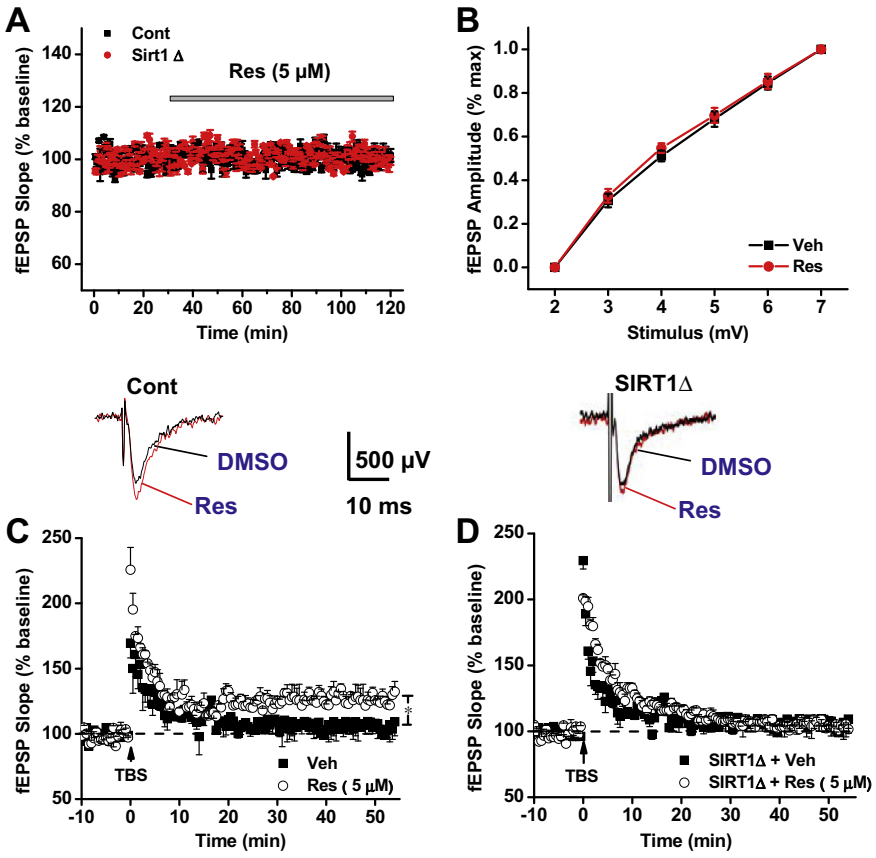

Fig. 2. Resveratrol regulates the plasticity in mouse hippocampus. (A) RSV treatment did not change the evoked fEPSP slope. (B) The amplitude of the evoked fEPSP in RSV treatment mice was similar as that in control mice (Veh). (C). LTP was induced by one TBS in the CA1 region from 8 to 9 month-old mice with or without RSV (5 μM) pretreatment for 1-h. By 40 min, the fEPSPs from Veh slice decayed to the baseline (n = 8 slices, 108.5 ± 3.2% compared with baseline) whereas fEPSPs from RSV treatment slice remained potentiated (n = 9 slices, 132.2 ± 7.8% compared with baseline). (D) The elevated effect of RSV on LTP in a SIRT1-dependent manner. Slices from *SIRT1*Δ mice failed to induce LTP under RSV treatment (n = 8 slice, 109.2 ± 10.7% compared with baseline).

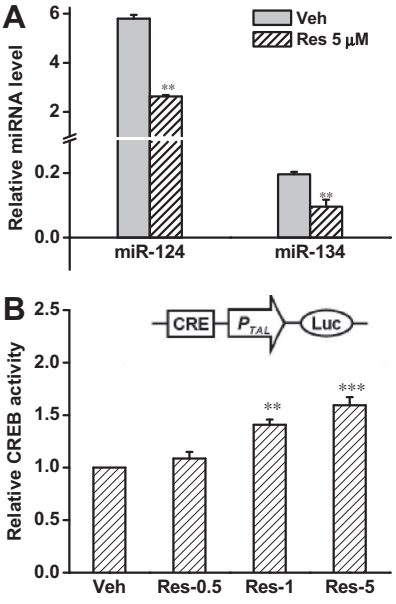

Fig. 3. Resveratrol up-regulates CREB-mediated transcriptional activity (A) RSV treatment decreased the levels of miR-124 and miR-134 in cultured hippocampus slice. (B) RSV dose-dependently upregulated CREB-mediated transcriptional activity. CAD cells transfected with a CREB activity reporter construct (CRE-Luc) were treated with different concentration RSV for 6-h. Reporter activities are displayed as average ± sem from multiple experiments (n = 3 per group).

observed in open-field test. Remarkably, the RSV mice showed markedly improved freezing behavior as evaluated by the context- and tone-dependent fear conditioning paradigm (p = 0.027, p = 0.0199, Fig. 1B) 24-h after training when compared to control mice. Mechanistically, we investigated whether the latter effect of RSV is mediated via SIRT1. Such a point is worthy of testing since there is also evidence that RSV may not be the direct agonist of SIRT1. The controversy was raised because the assay used by Howitz [19] to screen for small molecule activators of SIRT1 relied on a fluorescent substrate, and non-fluorescent assays have produced differing results [34,35]. Nevertheless, our present data indicated that the improved effect on memory of one-week RSV treatment was mediated by SIRT1 pathway. As shown in Fig. 1C, RSV did not enhance freezing behavior in *SIRT1*Δ mice, in contrast to control mice, indicating that the effects of RSV on learning and memory occur through SIRT1. Neither SIRT1 loss-of-function nor RSV treatment affected shock sensitivity (Fig. 1D). In addition, we examined whether the acetylation of PGC-1alpha was decreased following RSV treatment. Indeed, we observed a decrease in PGC-1alpha acetylation (Fig. 1A), suggesting that SIRT1 activity is increased following one-week RSV treatment. Although it is clear that the mode of action by RSV on SIRT1 in our system still need more investigation, the positive effect of RSV on learning and memory assayed in aged mice are encouraging, as it is consistent with a large body of literature pointing to the positive metabolic effects and potential therapeutic benefits of RSV administration.

Furthermore, we used the long-term potentiation (LTP) paradigm to directly determine the roles of RSV in synaptic plasticity.

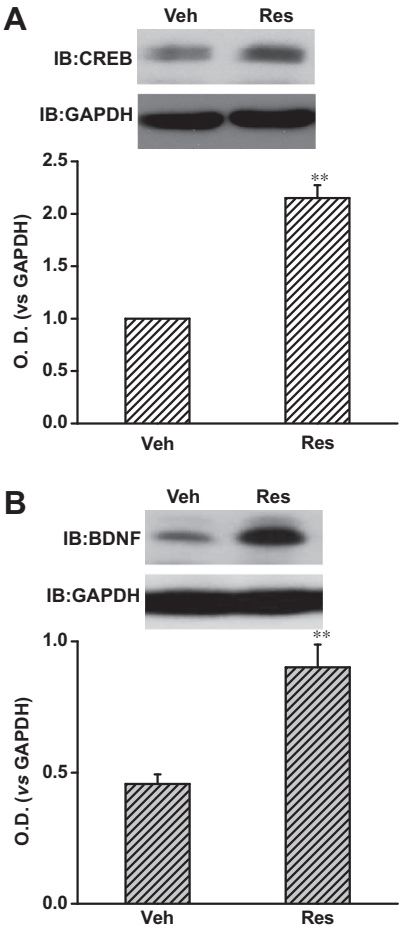

Fig. 4. Resveratrol treatment enhanced the expression levels of CREB and BDNF in cultured hippocampus slice. (A) CREB protein levels were elevated in the hippocampi of RSV mice. Band densities were normalized to GADPH. Representative Western blots are shown in upper panel. (B) Expression of BDNF protein levels in the hippocampi of RSV slice was increased compared with Veh mice. Hippocampi from mice ($n = 3$ per group) were dissected and subjected to Western blotting, and optical density is displayed as average ± sem. in the histogram. $^{**}p < 0.01$.

We performed electrophysiological recordings on hippocampal slices prepared from control mice subjected to one train of θ burst stimulation (TBS), which is insufficient to generate an LTP response in 8–9 month-old mice. However, 1-h pretreatment of RSV facilitated an LTP response from single train θ burst (Fig. 2C). Similar to the results seen from the functional measurements, RSV did not facilitate LTP in *SIRT1*Δ mice (Fig. 2D). Furthermore, field excitatory postsynaptic potential and paired pulse facilitation were not altered after RSV treatment (Fig. 2A and B), clearly ruling out the RSV effect of basic transmission. As our previous studies have suggested the role of miRNAs in regulating LTP, we analyzed the levels several brain specific miRNAs in RSV-treated hippocampus slices. Interestingly, the levels of miR-134 and 124 were down-regulated in RSV treated individuals. MiR-124 is one of the most conserved and abundantly expressed NS-specific miRNAs in brain and miR-134 is a brain-specific miR [36,37]. Both of them are reported to regulate CREB expression [22,26,27], which in turn regulates the activation of immediate early genes that ultimately facilitates synaptic plasticity. Taken together, these data indicate a positive relationship between RSV treatment and synapse plasticity, which can in turn impact learning and memory.

By orally administration, RSV is rapidly absorbed and readily metabolized to form mainly glucuronide and sulfate derivatives which are eliminated by urine [38,39]. It is generally believed that the effect of RSV on cell survival can be ambiguous and that it depends on the doses of RSV. One study analyzing the heart tissue showed that at lower doses of 2.5 or 5 mg/kg, RSV exerted survival signal by up-regulating the anti-apoptotic and redox proteins Akt and Bcl-2, while at higher doses (>25 mg/kg) it potentiated a death signal by down-regulating redox proteins and upregulating pro-apoptotic proteins [40]. In this study, we also observed the toxic effect of RSV at high dose ($\geqslant 50$ μM) on cultured neurons or cells (data not shown). In this regard, it is worth pointing out that although it is usually considered as an antioxidant, RSV can also exhibit pro-oxidant properties in the presence of transition metal ions such as copper, leading to oxidative breakage of cellular DNA [41]. Thus, while diet-rich antioxidants are generally beneficial for our health, we should still be careful to the dosage and frequency of usage.

Taken together, RSV treatment improved learning and memory in 8–9 month-old mice through miR-CREB-BDNF pathway. Our data are consistent with the large body of literature showing that the RSV-rich diet is generally beneficial for human health, and additionally suggest a new strategy for therapeutic intervention of human diseases associated with memory impairment.

Acknowledgments

We thank Dr. Li-Huei Tsai for *SIRT1*$^{\Delta ex4}$/Nestin-Cre mice, and Dr Jianghuai Liu for critical reading of the manuscript. This work is partially supported by the National Natural Science Foundations of China (Nos. 81222013, 30871264), National Basic Research Program of China (2013CB835100) and Specialized Research Funds for the Doctoral Program of Higher Education (20070284042, 20110091120029) to J. Gao.

References

[1] I. Cantuti-Castelvetri, B. Shukitt-Hale, J.A. Joseph, Neurobehavioral aspects of antioxidants in aging, Int. J. Dev. Neurosci. 18 (2000) 367–381.
[2] D. Harman, Free-radical theory of aging. Increasing the functional life span, Ann. NY Acad. Sci. 717 (1994) 1–15.
[3] R.G. Fariello, Biochemical profile of vulnerable neurons in neurodegenerative disorders, Int. J. Tissue React. 12 (1990) 179–181.
[4] J. Halder, A.N. Bhaduri, Protective role of black tea against oxidative damage of human red blood cells, Biochem. Biophys. Res. Commun. 244 (1998) 903–907.
[5] P. Saiko, A. Szakmary, W. Jaeger, T. Szekeres, Resveratrol and its analogs: defense against cancer, coronary disease and neurodegenerative maladies or just a fad?, Mutat Res. 658 (2008) 68–94.
[6] K.B. Harikumar, B.B. Aggarwal, Resveratrol: a multitargeted agent for age-associated chronic diseases, Cell Cycle 7 (2008) 1020–1035.
[7] J.A. Baur, D.A. Sinclair, Therapeutic potential of resveratrol: the *in vivo* evidence, Nat. Rev. Drug Discov. 5 (2006) 493–506.
[8] P. Kopp, Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'?, Eur J. Endocrinol. 138 (1998) 619–620.
[9] G.J. Soleas, E.P. Diamandis, D.M. Goldberg, Resveratrol: a molecule whose time has come? And gone?, Clin Biochem. 30 (1997) 91–113.
[10] D. Kim, M.D. Nguyen, M.M. Dobbin, A. Fischer, F. Sananbenesi, J.T. Rodgers, I. Delalle, J.A. Baur, G. Sui, S.M. Armour, P. Puigserver, D.A. Sinclair, L.H. Tsai, SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis, EMBO J. 26 (2007) 3169–3179.
[11] M. Das, D.K. Das, Resveratrol and cardiovascular health, Mol. Aspects Med. 31 (2010) 503–512.
[12] Y. Sakata, H. Zhuang, H. Kwansa, R.C. Koehler, S. Dore, Resveratrol protects against experimental stroke: putative neuroprotective role of heme oxygenase 1, Exp. Neurol. 224 (2010) 325–329.
[13] A. Dal-Pan, J. Terrien, F. Pifferi, R. Botalla, I. Hardy, J. Marchal, A. Zahariev, I. Chery, P. Zizzari, M. Perret, J.L. Picq, J. Epelbaum, S. Blanc, F. Aujard, Caloric restriction or resveratrol supplementation and ageing in a non-human primate: first-year outcome of the RESTRIKAL study in Microcebus murinus, Age (Dordr) 33 (2011) 15–31.
[14] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.
[15] A.P. Raval, K.R. Dave, M.A. Perez-Pinzon, Resveratrol mimics ischemic preconditioning in the brain, J. Cereb. Blood Flow Metab. 26 (2006) 1141–1147.

[16] S. Yousuf, F. Atif, M. Ahmad, N. Hoda, T. Ishrat, B. Khan, F. Islam, Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia, Brain Res. 1250 (2009) 242–253.
[17] J.A. Shin, H. Lee, Y.K. Lim, Y. Koh, J.H. Choi, E.M. Park, Therapeutic effects of resveratrol during acute periods following experimental ischemic stroke, J. Neuroimmunol. 227 (2010) 93–100.
[18] F. Zhang, Y. Wu, Resveratrol may be an effective prophylactic agent for ischemic stroke, J. Formos. Med. Assoc. 110 (2011) 485–486.
[19] K.T. Howitz, K.J. Bitterman, H.Y. Cohen, D.W. Lamming, S. Lavu, J.G. Wood, R.E. Zipkin, P. Chung, A. Kisielewski, L.L. Zhang, B. Scherer, D.A. Sinclair, Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan, Nature 425 (2003) 191–196.
[20] J.G. Wood, B. Rogina, S. Lavu, K. Howitz, S.L. Helfand, M. Tatar, D. Sinclair, Sirtuin activators mimic caloric restriction and delay ageing in metazoans, Nature 430 (2004) 686–689.
[21] W. Renthal, A. Kumar, G. Xiao, M. Wilkinson, H.E. Covington 3rd, I. Maze, D. Sikder, A.J. Robison, Q. LaPlant, D.M. Dietz, S.J. Russo, V. Vialou, S. Chakravarty, T.J. Kodadek, A. Stack, M. Kabbaj, E.J. Nestler, Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins, Neuron 62 (2009) 335–348.
[22] J. Gao, W.Y. Wang, Y.W. Mao, J. Graff, J.S. Guan, L. Pan, G. Mak, D. Kim, S.C. Su, L.H. Tsai, A novel pathway regulates memory and plasticity via SIRT1 and miR-134, Nature 466 (2010) 1105–1109.
[23] G.S. Liu, Z.S. Zhang, B. Yang, W. He, Resveratrol attenuates oxidative damage and ameliorates cognitive impairment in the brain of senescence-accelerated mice, Life Sci. 91 (2012) 872–877.
[24] R. Cristofol, D. Porquet, R. Corpas, A. Coto-Montes, J. Serret, A. Camins, M. Pallas, C. Sanfeliu, Neurons from senescence-accelerated SAMP8 mice are protected against frailty by the sirtuin 1 promoting agents melatonin and resveratrol, J. Pineal Res. 52 (2012) 271–281.
[25] D.J. Ho, N.Y. Calingasan, E. Wille, M. Dumont, M.F. Beal, Resveratrol protects against peripheral deficits in a mouse model of Huntington's disease, Exp. Neurol. 225 (2010) 74–84.
[26] P. Rajasethupathy, F. Fiumara, R. Sheridan, D. Betel, S.V. Puthanveettil, J.J. Russo, C. Sander, T. Tuschl, E. Kandel, Characterization of small RNAs in Aplysia reveals a role for miR-124 in constraining synaptic plasticity through CREB, Neuron 63 (2009) 803–817.
[27] J. Preethi, H.K. Singh, P.D. Charles, K. Emmanuvel Rajan, Participation of microRNA 124-CREB pathway: a parallel memory enhancing mechanism of standardised extract of Bacopa monniera (BESEB CDRI-08), Neurochem. Res. 37 (2012) 2167–2177.
[28] Y.S. Han, W.H. Zheng, S. Bastianetto, J.G. Chabot, R. Quirion, Neuroprotective effects of resveratrol against beta-amyloid-induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C, Br. J. Pharmacol. 141 (2004) 997–1005.
[29] P. Marambaud, H. Zhao, P. Davies, Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides, J. Biol. Chem. 280 (2005) 37377–37382.
[30] E. Savaskan, G. Olivieri, F. Meier, E. Seifritz, A. Wirz-Justice, F. Muller-Spahn, Red wine ingredient resveratrol protects from beta-amyloid neurotoxicity, Gerontology 49 (2003) 380–383.
[31] Q. Wang, S. Yu, A. Simonyi, G. Rottinghaus, G.Y. Sun, A.Y. Sun, Resveratrol protects against neurotoxicity induced by kainic acid, Neurochem. Res. 29 (2004) 2105–2112.
[32] N. Gacar, O. Mutlu, T. Utkan, I. Komsuoglu Celikyurt, S.S. Gocmez, G. Ulak, Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and Morris water maze tests in rats, Pharmacol .Biochem. Behav. 99 (2011) 316–323.
[33] S.S. Sahu, S. Madhyastha, G.M. Rao, Neuroprotective effect of resveratrol against prenatal stress induced cognitive impairment and possible involvement of Na(+), K(+)-ATPase activity, Pharmacol. Biochem. Behav. 103 (2013) 520–525.
[34] M.T. Borra, B.C. Smith, J.M. Denu, Mechanism of human SIRT1 activation by resveratrol, J. Biol. Chem. 280 (2005) 17187–17195.
[35] M. Kaeberlein, T. McDonagh, B. Heltweg, J. Hixon, E.A. Westman, S.D. Caldwell, A. Napper, R. Curtis, P.S. DiStefano, S. Fields, A. Bedalov, B.K. Kennedy, Substrate-specific activation of sirtuins by resveratrol, J. Biol. Chem. 280 (2005) 17038–17045.
[36] M. Lagos-Quintana, R. Rauhut, A. Yalcin, J. Meyer, W. Lendeckel, T. Tuschl, Identification of tissue-specific microRNAs from mouse, Curr. Biol. 12 (2002) 735–739.
[37] G.M. Schratt, F. Tuebing, E.A. Nigh, C.G. Kane, M.E. Sabatini, M. Kiebler, M.E. Greenberg, A brain-specific microRNA regulates dendritic spine development, Nature 439 (2006) 283–289.
[38] T. Walle, Bioavailability of resveratrol, Ann. NY Acad. Sci. 1215 (2011) 9–15.
[39] C.H. Cottart, V. Nivet-Antoine, C. Laguillier-Morizot, J.L. Beaudeux, Resveratrol bioavailability and toxicity in humans, Mol. Nutr. Food Res. 54 (2010) 7–16.
[40] J. Dudley, S. Das, S. Mukherjee, D.K. Das, Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose, J. Nutr. Biochem. 20 (2009) 443–452.
[41] C.A. de la Lastra, I. Villegas, Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications, Biochem. Soc. Trans. 35 (2007) 1156–1160.